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Developing anti-inflammatory therapeutics for patients with osteoarthritis

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Abstract

Osteoarthritis (OA) is the most common joint disorder in the world but there are no approved therapeutics to prevent disease progression. Historically, OA has been considered a wear and tear joint disease and efforts to identify and develop disease modifying therapeutics have predominantly focussed on direct inhibition of cartilage degeneration. However, there is now increasing evidence that inflammation is a key mediator of OA joint pathology and also that the link between obesity and OA is not solely due to excessive load-bearing, suggesting therefore that targeting inflammation in OA could be a rewarding therapeutic strategy. In this review we therefore re-evaluate historical clinical trial data on anti-inflammatory therapeutics in OA patients, highlight some of the more promising emerging therapeutic targets and discuss the implications for future clinical trial design.

Osteoarthritis and inflammation

Inflammation in OA has been overlooked for many years.. However, histological analysis, ultrasound and MRI imaging have shown evidence of synovitis in OA joints [1] with increased cellular infiltration of activated B cells and T lymphocytes. Histological studies indicate that synovitis is present in 50% of patients with early OA, and in nearly all patients with late-stage OA [2]. Furthermore, synovial hypertrophy as detected by ultrasound has been shown to correlate to knee OA disease severity [3].

Several studies have reported elevated levels of a number of pro-inflammatory mediators (including TNF- α , IL-1 β , IL-6, IL-15, IL-17, IL-18 and NO) in the serum or synovial fluid of OA patients [4, 5], compared to healthy patients (Figure 1). Furthermore, stimulation of cartilage tissue with pro-inflammatory cytokines mimics many of the structural changes associated with OA [6-8].

In light of this , it is perhaps surprising that clinical studies in OA patients with anti-inflammatory therapeutics have thus far disappointed. However, OA pathology is highly heterogeneous, with the degree of synovitis varying amongst patients. Indeed, synovitis is not present in half of patients with early OA [2]. Therefore, patient selection is an important consideration for such clinical studies, and unless taken into account could impact on whether the clinical end point is met. For this reason, we re-evaluate the clinical trial data of anti-inflammatory therapeutics in OA (Table 1), to highlight in particular the specific OA patient cohort studied, whether patients were selected for synovitis and the clinical end-points measured. This is not a systematic review. Instead, we have focussed on completed randomised control

63 trials (RCTs) conducted during the last 10 years in patients with hand, knee and hip
64 OA, which have reported clinical end-points. Secondly, we explore the
65 opportunities for the development of new targeted therapeutics (Table 2) and discuss
66 the requirement to stratify patients for such approaches.

68 **Anti-inflammatory therapeutics in OA patients**

69 ***Anti TNF- α therapies***

70 In OA joint tissues, synovial fluid, and serum, TNF- α has been reported to be
71 elevated compared to healthy patients [9-11]. In rodent models of traumatic joint
72 injury, TNF- α expression is induced [12] and correlates to joint space narrowing
73 (JSN) [13]. Furthermore, overproduction of TNF- α induces NO [14], and upregulates
74 the expression of MMPs [15], whilst TNF- α receptor antagonists block NO production
75 in human cartilage tissue [16]. TNF- α has also been demonstrated to inhibit
76 proteoglycan [17] and Type II collagen synthesis. However, despite these data and
77 the proven successes in treating rheumatoid arthritis (RA) patients with anti-TNF- α
78 therapeutics, clinical trials in OA patients have yielded conflicting and generally
79 disappointing results.

81 Adalimumab (Humira; Abbott Laboratories, IL) is a human monoclonal antibody
82 (mAb) bioengineered to bind to TNF- α and prevent receptor binding [18]. It has been
83 shown to be efficacious in reducing the pathological symptoms of RA in several
84 clinical trials^(DE001/003, DE004, DE007, DE009, and DE010) [18]. However, its effect on OA
85 disease modification has not been clinically proven.

In a PhII, 12 month RCT^(NCT00296894), 60 patients with active erosive hand OA received Adalimumab (40mg) or placebo subcutaneously every two weeks. Progression from palpable soft tissue swelling to joint damage decreased 10-fold in the Adalimumab group compared to placebo [19], with a reduction in the proportion of patients experiencing a new erosive interphalangeal joint at 12 month follow-up (26.7%vs 40% placebo control), However these findings did not reach statistical significance and Adalimumab was deemed ineffective at reducing disease activity [19].

In a PhI/II study of 17 patients with knee OA^(NCT00686439), Adalimumab (40mg, every 2 weeks) resulted in significant improvements in joint pain at 12 weeks with WOMAC 20% and WOMAC 50% responses in 70% and 40% of patients respectively [20]. However, this study did not incorporate measures of disease modification including JSN, so it is unknown whether prolonged treatment with Adalimumab in this patient cohort would ultimately be disease-modifying. Furthermore, Adalimumab failed to reach its primary end-point of improving pain in a PhII study^(NCT00597623) in patients with hand OA refractory to standard-of-care analgesics [21].

It is clear that anti-TNF- α therapeutics are far less efficacious in improving OA pain and joint mobility compared to the outcomes achieved in RA patients. Indeed, RA is a more inflammatory condition with greater evidence of TNF- α and immune cell involvement. However, some positive results have been reported with Adalimumab in patients with knee OA and in patients with erosive hand OA. It should be stressed that these clinical studies did not measure disease progression, and likely involved highly heterogeneous OA patient cohorts. Despite several pilot studies^(NCT01144143)

[22], and small RCTs ^(NCT00819572) [23] , larger placebo RCTs to determine the efficacy of other anti-TNF- α therapies such as infliximab and DLX105 (TNF- α mAb) have not yet been undertaken to fully evaluate the potential of TNF- α therapeutics in OA [22, 24].

IL-1 β signalling inhibitors

IL-1 β has been purported to be a driver of OA pathology [25] by mediating damage to articular cartilage tissue [6, 26, 27] and inhibiting the anabolic processes of articular chondrocytes [28]. Found in its active form in cartilage, synovial fluid and synovium, IL-1 β inhibits type II collagen [8], aggrecan [8] and proteoglycan synthesis [29] and stimulates production of pro-inflammatory cytokines, MMPs and PGE2. Increased abundance of IL-1 β has been reported in synovial fluid of both knee OA and ACL patients, compared to healthy individuals [30-33], and in the synovial membrane expression correlates with OA grade [9].

Diacerein, a small molecule IL-1 β inhibitor, reduces the number of IL-1 receptors resulting in a reduction in functional IL-1 heterodimer receptor complexes [34]. It has demonstrated “anti-arthritic” properties both *in vitro* [35] and *in vivo* in a murine model [36] through the inhibition of MMPs [37] and NO production [38]. In a 3yr RCT^(NCT00451360), 507 hip OA patients received either Diacerein or placebo (2x50mg orally) daily. Although pain and functional impairment associated with OA remained unchanged, Diacerein significantly reduced JSN compared to placebo [39]. However, gastrointestinal adverse effects with Diacerein were a common, cause of treatment discontinuation, restricting its usage. It is not recommended for individuals aged over 65 years.

Alternative IL-1 pathway inhibitors such as the human mAb AMG108 have been designed to directly target the IL-1 receptor. In a RCT^(NCT00110942) of 160 patients with knee OA, AMG108 was well tolerated independent of dosage or route of administration. However, although marginal improvements in pain were noted in the AMG108 group, the difference was statistically insignificant and the clinical endpoint was not achieved [40].

Given the limited clinical efficacy with subcutaneous administration of IL-1 β inhibitors and the potential for adverse systemic events, recent studies have focussed on intra-articular (I.A) administration [41]. A RCT^(NCT00110916) involving 170 patients with painful knee OA administered either 50mg or 150mg of Anakinra (a IL-1 β receptor antagonist), or placebo control. Unfortunately, no improvement in WOMAC score or cartilage turnover was reported after 4 weeks [42]. However, patients were not pre-selected on the basis of any confirmed synovitis. Thus it is possible that the study population contained a heterogeneous mix of responders and non-responders.

Arachidonic acid pathway inhibitors

Non-steroidal anti-inflammatory drugs (NSAIDs) are often prescribed to reduce OA pain [43]. Pharmacologically they inhibit cyclooxygenase (COX) enzymes in the arachidonic acid pathway, leading to a reduction in prostaglandin synthesis, which are known mediators of pain and inflammation. Non-selective NSAIDs (nsNSAIDs) (e.g Ibuprofen, naproxen and Diclofenac), inhibit both COX-1 and COX-2 enzymes, and their efficacy in reducing pain, inflammation and swelling in patients with OA has been demonstrated in several clinical studies^(NCT01461369, NCT01860833) [44-46].

Critically, long-term administration of nsNSAIDs is associated with significant adverse events from GI perforation, ulceration and bleeding [47] due to the reduction of GI tract protective prostanoids; a process mediated by COX-1 [48]. Due to these issues, selective COX-2 inhibitors have been developed, including Celecoxib and Valdecoxib.

In a RCT^(NCT00650624), Valdecoxib (5mg, 10mg, and 20mg daily dosage) was compared to the nsNSAID naproxen (500mg twice daily dosage), in patients with moderate/severe knee OA. At 5mg and 10mg doses, the Valdecoxib group exhibited significantly reduced GI toxicity compared to the naproxen group [49]. Unfortunately, although clinical trials demonstrated a reduction in GI complications [50, 51], an increased risk of adverse cardiovascular events were noted [52]. This is attributed to the mode-of-action of selective COX-2 inhibitors which decrease prostacyclin production without altering thromboxane levels.

An alternative approach is dual inhibition of leukotrienes and prostaglandins, by targeting both 5-lipoxygenase (LOX) activity and COX-2. One such COX/LOX inhibitor is Licofelone (Merckle GmbH). In preclinical models of OA, Licofelone has shown disease-modifying efficacy in reducing synovium hypertrophy, cartilage lesions and production of pro-inflammatory PGE2 and leukotrienes B4 [53, 54].

Licofelone was shown to significantly reduce cartilage volume loss (vs nsNSAID alone) in a recent RCT of patients with knee OA [55]. Specifically, 355 knee OA patients received twice daily naproxen (500mg) or licofelone (200mg). Cartilage

187 volume and JSN was measured at baseline, 6, 12 and 24 months [55]. Global
188 cartilage loss was significantly less in the licofelone group compared with naproxen
189 [55]. Critically, no reported GI or CV toxicity at efficacious concentrations were
190 recorded [56].

191
192 Despite these promising results, Licofelone has not yet been submitted for regulatory
193 approval. There is evidence to suggest that the rate of OA progression may increase
194 in patients receiving long-term NSAID treatment. Radiological assessment of knee
195 and hip OA progression found an increase in the risk of OA progression in
196 individuals on long-term Diclofenac treatment (>180 days) compared to individuals
197 on short-term treatment (1-30 days) [57]. Indeed, in contrast to their efficacy in
198 blocking pro-inflammatory responses, COX2 inhibitors have been reported to delay
199 the resolution of inflammation in chronic inflammatory preclinical models [58].
200 Whether these data are the reason why Licofelone has not progressed since its
201 positive PhIII data is unclear.

202 203 **NO inhibitors**

204 NO plays an important role in inflammatory processes and is considered a key
205 mediator of cartilage destruction in OA [59]. NO and the inducible NO synthase
206 (iNOS) are upregulated in preclinical animal models of OA [60] as well as in human
207 OA chondrocytes [61], and mediate the release of pro-inflammatory cytokines.
208 Indeed, NO can mediate many of the pathogenic effects of IL-1 β through the
209 activation of MMPs, the inhibition of proteoglycan and collagen synthesis and the
210 enhancement of inflammation [62]. Preclinical studies have shown that iNOS KO
211 mice are resistant to development of OA [63], and that pharmacological inhibition of

iNOS reduces OA progression and pain in the MIA rodent of OA [64] and in a canine model of OA [65] .

Aa recent clinical trial investigated the safety and efficacy of a novel irreversible iNOS inhibitor (SD-6010) on slowing OA progression in a cohort of overweight and obese patients with knee OA^(NCT00565812). Disappointingly, although the iNOS inhibitor was well tolerated, the drug failed to slow the rate of JSN versus placebo over a course of 96 weeks [66]. However, additional iNOS inhibitors are in development, including S-Methylisothiurea, which in preclinical models is effective in reducing NO and disease progression in the MIA rodent model of OA [67]. Whether this promising preclinical data translates into human efficacy is a critical question, but these are encouraging studies.

Emerging anti-inflammatory drug targets

Since efforts to develop effective disease modifying OA therapeutics against prime inflammatory drug targets have yet to bear fruit, ongoing research to better understand the inflammatory response in OA joint tissues is critical so that new drug targets can be identified. There are a number of different therapeutic approaches being investigated which may ultimately provide an effective anti-inflammatory therapeutic for OA patients, including non-pharmacological approaches such as nutraceuticals and gene therapy [68]. Below we summarise some of the promising emerging targets, based on their disease linkage to OA and available preclinical data.

Synovitis associated cytokines

In addition to TNF- α and IL-1 β , many additional cytokines and chemokines, detectable in both the synovial fluid and in serum, have also been associated with OA [69]. One notable example is IL-15 which has been associated with RA pathology. However, IL-15 may also play an important role in early OA, since IL-15 expression in synovial fluid [70], and in serum are associated with the progression of early knee OA [71] and pain [72]. Similarly, IL-7 has been implicated in joint inflammation [73] since studies have shown it can mediate TNF- α production in the RA joint [74]. Importantly, recent studies have shown that IL-7 is expressed by articular chondrocytes and that IL-7 stimulation of cartilage induces MMP13 production and proteoglycan loss [75].

Critically, whether these inflammation-associated cytokines are central regulators of early OA disease pathology or purely biomarkers of an inflammatory tissue is an important question. This will require target validation studies in appropriate and translatable preclinical models, as well as in human *ex-vivo* tissues. One of the prime candidates for such studies is IL-17 since IL-17A polymorphisms correlate to OA susceptibility in particular patient cohorts [76]. In knee OA, IL-17 is elevated in both the serum and synovial fluid, and correlates with KL grade [77]. *In vitro* human chondrocyte studies have shown IL-17 stimulates the production of NO and MMPs [78], and decreases TIMP-2 and TIMP4 expression [79]. Given these data, studies that aim to modulate IL-17 expression or its signalling activity in appropriate models are an important next step.

p38 pathway inhibitors

There is good evidence that the p38 MAPK signalling pathway is activated in OA, and is a prominent pathway which mediates pro-inflammatory cytokine signal transduction. In human OA articular cartilage, p38 is phosphorylated by several putative drivers of OA including IL-1 β , TNF α and damage associated molecular patterns (DAMPs) such as fibronectin fragments [80, 81]. Furthermore, p38 inhibitors have shown efficacy in reducing cartilage degeneration in both the MIA OA model [82-84], and reducing pain in RA animal models [85].

Clinically, a p38 inhibitor (PH-797804) is currently being tested for its analgesic efficacy when administered orally in patients with knee OA KL grade <2 ^(NCT01102660). However, due to the cell-type-dependent pro-inflammatory and anti-inflammatory functions of p38 MAPK [86], systemic delivery of p38 inhibitors may not be appropriate. An alternative approach is to develop therapeutics that target molecules downstream in the p38 MAPK pathway, thus providing a more selective targeting approach.

MAPKAPK2 (MK2) is one of several kinases directly activated by p38 [87], and is known to post-transcriptionally regulate transcripts containing adenine rich elements [88] including TNF- α and MMP-13. We have previously shown that MK2 is active in areas of cartilage damage, and that MK2 inhibition is as effective as p38 inhibition in preventing the IL-1 β -mediated secretion of PGE2 and MMPs [89]. The *in vivo* efficacy of MK2 inhibition in OA preclinical models has not yet been reported, but MK2 inhibitors have shown efficacy in an inflammatory bowel murine model [90].

Toll-like receptors

Several DAMPs are associated with OA including cartilage extracellular matrix fragments and plasma proteins that enter the synovial fluid. Binding of DAMPs to pattern recognition receptors such as Toll-like receptors (TLRs) can activate NF κ B-mediated inflammatory signalling. Articular chondrocytes have been shown to express TLR1-9 [91], but studies on TLR4 have so far provided the strongest link to OA. In humans, TLR4 expression is associated with areas of cartilage damage [92], JSN [93], and the expression of pro-inflammatory cytokines including IL-1 β , TNF α , PGE₂ and NO [94]. Multiple agents that either bind to TLR4 agonists (e.g Pep-1), or modulate TLR4 signalling (e.g PPAR δ and BMP-7) can inhibit pro-inflammatory and pro-catabolic effects in chondrocytes [91, 95] and prevent cartilage loss in a murine model of OA [96]. Therefore, development of specific pharmacological TLR4 inhibitors may be of therapeutic value.

A number of studies have also examined the role of TLR2 in OA. Similarly to TLR4, its expression is associated with lesional areas of cartilage damage [92], and several reports have demonstrated that TLR2 stimulation induces the production of pro-inflammatory cytokines [97-99] and cartilage degradation [92]. However, *in vivo* modulation of TLR2 in arthritic joint models has revealed conflicting results. Of concern, TLR2 knockout mice develop a more severe OA pathology [100], which could be due to compensatory developmental effects which have yet to be established. More promisingly, TLR2 mAbs have been shown to inhibit both the production of pro-inflammatory cytokines and reduce the development of arthritis in the collagen-induced arthritis (CIA) model [99]. The therapeutic utility of such TLR2 mAbs in OA specific models has not yet been reported.

Adipose secreted cytokines

The discovery that adipose tissue secretes cytokines (adipokines) has led to the current understanding of adipose tissue as an endocrine organ [101]. Adipokine release has been mechanistically linked to metabolic complications and the metabolic syndrome [102, 103] by contributing to the low-level pro-inflammatory state seen commonly in obese individuals [104].

Critically, adipokine signalling is now receiving much attention in relation to OA joint pathophysiology largely due to the association between obesity and OA [104, 105] in both weight-bearing and non-weight bearing joints (e.g hands) [106]. Furthermore, differential expression of particular adipokines have been reported in OA serum and synovial fluid, which are capable to modulating cartilage catabolic and anabolic pathways [105].

One of the most widely studied adipokines is leptin, a product of the *ob* gene, which is known to regulate metabolism and appetite [107]. Leptin is increased in OA synovial fluid compared to non-OA controls [108]. Recombinant leptin, either alone or synergistically with IL-1 β , induces MMP-1 and MMP-13 expression in primary human chondrocytes [109] and increases the production of inflammatory mediators including IL-1 β , IL-6, IL-8 and PGE2 [110]. Due to the pro-inflammatory nature of leptin and its potential to drive cartilage catabolism, the recent development of peptide-based and antibody-based leptin antagonists could provide an effective therapeutic [111, 112].

RNAi based therapeutics

RNA interference (RNAi)-based therapeutics such as siRNAs represents a relatively new and largely untapped avenue of therapeutic potential [113]. Relatively cheap to design and produce, this class of therapeutic is highly attractive to drug discovery [114].

One of the greatest hurdles facing siRNA therapeutics is achieving successful *in vivo* delivery to the site of action. Unless protected, for example by complexing with cationic lipids, siRNAs rapidly degrade in serum [115]. Successful preclinical studies using naked siRNAs have predominantly been achieved where systemic delivery can be avoided, such as in airway disease models [116], or in ocular conditions where the siRNA can be delivered directly to the site of action [117].

Systemic *in vivo* delivery of anti-inflammatory siRNAs has produced positive results in preclinical models of inflammatory joint disease where cationic lipids have been utilised as delivery vehicles. For example, reduced arthritis disease severity has been reported in the CIA murine RA model using cationic lipids to deliver siRNA targeting TNF α [118] and siRNA targeting a panel of pro-inflammatory mediators including TNF α , IL-1 β , IL-6, IL-18 and cytosolic phospholipase A $_2$ α [119]. In addition, IA delivery of virally packaged siRNAs targeting TNF α have shown disease-modifying efficacy in the murine CIA RA model and similarly siRNA targeting NF κ B in the rodent meniscus tear OA model [120].

Optimisation of the delivery of RNAi-based therapeutics into the joint is required, together with a greater understanding of their specificity before their utility as OA

therapeutics can be fully realised. However, given that a limited proportion of targets are considered tractable with either small molecule or mAb entities, this field represents a highly important one. Furthermore, since Next Generation Sequencing has now identified multiple families of non-coding RNAs (e.g microRNA and LincRNAs) [121], the number of potential targets for therapeutic intervention is set to markedly increase. Importantly, several of these non-coding RNAs have already been shown to regulate chondrocyte inflammatory responses.

Stratification of OA patients for anti-inflammatory therapeutics

Critically, for clinical trials of anti-inflammatory therapeutics, synovitis is not reported to be present in all patients with early OA. However, one cohort where synovitis is believed to play a central role is post- traumatic OA, which is attributed to 12% of symptomatic OA of the hip, knee and ankle [122]. Electron microscopy analysis suggests that trauma induces a differential synoviocyte configuration compared to primary OA synovitis [123], and increased cytokine expression has been documented after joint trauma in chondrocytes, synoviocytes, infiltrating immune cells, and synovial fluid [30, 124, 125].

Gender may also be an important factor to consider for trials of particular OA anti-inflammatory therapeutics. There are no large cohort studies that have reported differences in the degree of synovitis between male and female patients with OA using modern imaging techniques. However, such imaging techniques cannot determine differences in individual inflammatory mediators. For example, free levels of leptin have been reported to be higher in the joints of females with knee OA,

385 compared to males [126]. This finding could explain why the association between
386 obesity and OA risk is reported to be greater in females [127], and suggests that
387 therapeutics targeting leptin signalling could be more efficacious in female OA
388 patients. Furthermore, a longitudinal prospective follow-up study found that females
389 exhibited a greater incidence of poly-articular OA, more rapid structural progression,
390 and a more severe symptomatic disease [128]. The higher incidence of multi-joint in
391 females with hip OA could indicate the presence of a systemic inflammatory OA
392 driver which is not present to the same degree, in males [128]. In addition, the
393 increase in incidence of OA in females rises dramatically post-menopause [129,
394 130], with retrospective case-controlled studies demonstrating a protective and anti-
395 inflammatory role of estrogen in OA [131-133]. Indeed, *in vitro* studies have shown
396 that estrogens enhance glucosaminoglycan synthesis [134], inhibit COX2 mRNA
397 expression [135] and protect against TNF- α , oncostatin-M [136], and ROS induced
398 damage [137]. Furthermore, modulation of estrogen signalling is itself a potential
399 pipeline for developing a therapeutic. However, estrogen receptor α or β agonists
400 have failed to demonstrate anti-inflammatory efficacy in patients with RA [138].

401
402 This knowledge of the diverse OA patient population necessitates the requirement
403 for clear patient stratification in clinical trials. The impact of not selecting the
404 appropriate patient population was illustrated by clinical trials with the drug Iressa
405 (AstraZeneca), an EGFR-targeted therapy for non-small cell lung cancer patients.
406 Iressa originally failed to meet its clinical end point during a PhII trial. However,
407 subsequent analysis showed those patients with EGFR mutation [139] had improved
408 survival. Not selecting the appropriate patient population masked the beneficial
409 effect of the drug in the original clinical study. Iressa was eventually approved 5

years after the original PhIII trial and several large costly follow-up clinical studies [139]. An important step towards identifying such clinical patient-selection biomarkers for clinical trials of OA anti-inflammatory therapeutics was recently made by Ruston and colleagues who identified a subgroup of hip OA patients that are epigenetically and transcriptomically predisposed to a cartilage inflammatory phenotype due to demethylation of the promoters of inflammatory/immunity-related genes [140].

Conclusions

Increasing evidence that inflammation contributes to OA joint pathology, particularly in specific patient cohorts, places great importance on both the identification of new drug targets that mediate inflammation in the joint and the requirement to re-evaluate historical clinical trial data. Preclinical studies have identified several promising targets for potential therapeutic development, and further opportunities will likely arise in the near future from new drug classes (such as RNAi therapeutics) and from the re-purposing of candidate drugs from other therapeutic research areas.

Critical in the development of any future anti-inflammatory therapeutic will be the need to identify and select the appropriate patient cohort. Our review of the historical clinical trial data of anti-inflammatory therapeutics in OA patients reveals that trials have not selected or stratified patients on the basis of synovitis or other inflammatory biomarker measurements. As such, some of these reagents may be worth revisiting in the clinic in the context of an appropriately selected patient cohort. Ideally in such cases a specific genomic or proteomic patient-selection biomarker would be identified closely linked to the mechanism of action of the therapeutic being tested. In

the absence of such a specific biomarker, it would appear pertinent to select patients on the basis of overt synovitis from either MRI or imaging analysis of the joint. Such an approach will result in smaller and cheaper clinical trials, with a much greater chance to observe beneficial effects, and ultimately is the route to the development of a personalised OA therapeutic.

Key Messages

- 1) Inflammation is a contributor to OA joint pathology, particularly in specific patient cohorts.
- 2) Preclinical studies have identified several promising targets for potential therapeutic development.
- 3) Clinical trials have not detailed any patient selection based on synovitis or inflammatory biomarker measurement.

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Figure 1 was produced using Omnigraffle 6. Clinical trials were identified using www.clinicaltrials.gov.

Disclosure statement

The authors declare no conflict of interest

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References

1. Fernandez-Madrid F, Karvonen RL, Teitge RA, Miller PR, An T, Negendank WG: **Synovial thickening detected by MR imaging in osteoarthritis of the knee confirmed by biopsy as synovitis.** *Magnetic resonance imaging* 1995, **13**(2):177-183.
2. Haywood L, McWilliams DF, Pearson CI, Gill SE, Ganesan A, Wilson D, Walsh DA: **Inflammation and angiogenesis in osteoarthritis.** *Arthritis Rheum* 2003, **48**(8):2173-2177.
3. Hall M, Doherty S, Courtney P, Latief K, Zhang W, Doherty M: **Synovial pathology detected on ultrasound correlates with the severity of radiographic knee osteoarthritis more than with symptoms.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2014, **22**(10):1627-1633.
4. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H: **Role of proinflammatory cytokines in the pathophysiology of osteoarthritis.** *Nat Rev Rheumatol* 2011, **7**(1):33-42.
5. Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B: **Synovial tissue inflammation in early and late osteoarthritis.** *Annals of the rheumatic diseases* 2005, **64**(9):1263-1267.
6. Kobayashi M, Squires GR, Mousa A, Tanzer M, Zukor DJ, Antoniou J, Feige U, Poole AR: **Role of interleukin-1 and tumor necrosis factor alpha in matrix degradation of human osteoarthritic cartilage.** *Arthritis and rheumatism* 2005, **52**(1):128-135.
7. Jones SW, Watkins G, Le Good N, Roberts S, Murphy CL, Brockbank SM, Needham MR, Read SJ, Newham P: **The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2009, **17**(4):464-472.
8. Stove J, Huch K, Gunther KP, Scharf HP: **Interleukin-1beta induces different gene expression of stromelysin, aggrecan and tumor-necrosis-factor-stimulated gene 6 in human osteoarthritic chondrocytes in vitro.** *Pathobiology* 2000, **68**(3):144-149.
9. Smith MD, Triantafillou S, Parker A, Youssef PP, Coleman M: **Synovial membrane inflammation and cytokine production in patients with early osteoarthritis.** *The Journal of rheumatology* 1997, **24**(2):365-371.
10. Hulejova H, Baresova V, Klezl Z, Polanska M, Adam M, Senolt L: **Increased level of cytokines and matrix metalloproteinases in osteoarthritic subchondral bone.** *Cytokine* 2007, **38**(3):151-156.
11. Amin AR: **Regulation of tumor necrosis factor-alpha and tumor necrosis factor converting enzyme in human osteoarthritis.** *Osteoarthritis Cartilage* 1999, **7**(4):392-394.
12. Aktas E, Sener E, Zengin O, Gocun PU, Deveci MA: **Serum TNF-alpha levels: potential use to indicate osteoarthritis progression in a mechanically induced model.** *Eur J Orthop Surg Tr* 2012, **22**(2):119-122.
13. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, Ding C: **Circulating levels of IL-6 and TNF-alpha are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults.** *Osteoarthritis and Cartilage* 2010, **18**(11):1441-1447.
14. McInnes IB, Leung BP, Field M, Wei XQ, Huang FP, Sturrock RD, Kinninmonth A, Weidner J, Mumford R, Liew FY: **Production of nitric oxide in the synovial membrane of rheumatoid and osteoarthritis patients.** *The Journal of experimental medicine* 1996, **184**(4):1519-1524.
15. Dayer JM, Beutler B, Cerami A: **Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts.** *The Journal of experimental medicine* 1985, **162**(6):2163-2168.
16. Vuolteenaho K, Moilanen T, Hamalainen M, Moilanen E: **Effects of TNFalpha-antagonists on nitric oxide production in human cartilage.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2002, **10**(4):327-332.
17. Saklatvala J: **Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage.** *Nature* 1986, **322**(6079):547-549.

18. Rau R: **Adalimumab (a fully human anti-tumour necrosis factor alpha monoclonal antibody) in the treatment of active rheumatoid arthritis: the initial results of five trials.** *Annals of the rheumatic diseases* 2002, **61 Suppl 2**:ii70-73.
19. Verbruggen G, Wittoek R, Vander Cruyssen B, Elewaut D: **Tumour necrosis factor blockade for the treatment of erosive osteoarthritis of the interphalangeal finger joints: a double blind, randomised trial on structure modification.** *Annals of the rheumatic diseases* 2012, **71(6)**:891-898.
20. Maksymowych WP, Russell AS, Chiu P, Yan A, Jones N, Clare T, Lambert RG: **Targeting tumour necrosis factor alleviates signs and symptoms of inflammatory osteoarthritis of the knee.** *Arthritis research & therapy* 2012, **14(5)**:R206.
21. Chevalier X, Ravaud P, Maheu E, Baron G, Rialland A, Vergnaud P, Roux C, Maugars Y, Mulleman D, Lukas C *et al*: **Adalimumab in patients with hand osteoarthritis refractory to analgesics and NSAIDs: a randomised, multicentre, double-blind, placebo-controlled trial.** *Annals of the rheumatic diseases* 2014.
22. Fioravanti A, Fabbroni M, Cerase A, Galeazzi M: **Treatment of erosive osteoarthritis of the hands by intra-articular infliximab injections: a pilot study.** *Rheumatology international* 2009, **29(8)**:961-965.
23. Guler-Yuksel M, Allaart CF, Watt I, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, van Schaardenburg D, van Krugten MV, Dijkmans BA, Huizinga TW, Lems WF *et al*: **Treatment with TNF-alpha inhibitor infliximab might reduce hand osteoarthritis in patients with rheumatoid arthritis.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2010, **18(10)**:1256-1262.
24. Magnano MD, Chakravarty EF, Broudy C, Chung L, Kelman A, Hillygus J, Genovese MC: **A pilot study of tumor necrosis factor inhibition in erosive/inflammatory osteoarthritis of the hands.** *Journal of Rheumatology* 2007, **34(6)**:1323-1327.
25. Daheshia M, Yao JQ: **The interleukin 1beta pathway in the pathogenesis of osteoarthritis.** *The Journal of rheumatology* 2008, **35(12)**:2306-2312.
26. Fan Z, Bau B, Yang H, Soeder S, Aigner T: **Freshly isolated osteoarthritic chondrocytes are catabolically more active than normal chondrocytes, but less responsive to catabolic stimulation with interleukin-1beta.** *Arthritis and rheumatism* 2005, **52(1)**:136-143.
27. Pfander D, Heinz N, Rothe P, Carl HD, Swoboda B: **Tenascin and aggrecan expression by articular chondrocytes is influenced by interleukin 1beta: a possible explanation for the changes in matrix synthesis during osteoarthritis.** *Annals of the rheumatic diseases* 2004, **63(3)**:240-244.
28. Goldring MB, Fukuo K, Birkhead JR, Dudek E, Sandell LJ: **Transcriptional suppression by interleukin-1 and interferon-gamma of type II collagen gene expression in human chondrocytes.** *J Cell Biochem* 1994, **54(1)**:85-99.
29. Nietfeld JJ, Wilbrink B, Den Otter W, Huber J, Huber-Bruning O: **The effect of human interleukin 1 on proteoglycan metabolism in human and porcine cartilage explants.** *J Rheumatol* 1990, **17(6)**:818-826.
30. Marks PH, Donaldson ML: **Inflammatory cytokine profiles associated with chondral damage in the anterior cruciate ligament-deficient knee.** *Arthroscopy* 2005, **21(11)**:1342-1347.
31. Bigoni M, Sacerdote P, Turati M, Franchi S, Gandolla M, Gaddi D, Moretti S, Munegato D, Augusti CA, Bresciani E *et al*: **Acute and late changes in intraarticular cytokine levels following anterior cruciate ligament injury.** *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* 2013, **31(2)**:315-321.
32. McNulty AL, Rothfusz NE, Leddy HA, Guilak F: **Synovial fluid concentrations and relative potency of interleukin-1 alpha and beta in cartilage and meniscus degradation.** *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* 2013, **31(7)**:1039-1045.

33. Kubota E, Imamura H, Kubota T, Shibata T, Murakami K: **Interleukin 1 beta and stromelysin (MMP3) activity of synovial fluid as possible markers of osteoarthritis in the temporomandibular joint.** *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons* 1997, **55**(1):20-27; discussion 27-28.
34. Martel-Pelletier J, Pelletier JP: **Effects of diacerein at the molecular level in the osteoarthritis disease process.** *Ther Adv Musculoskelet Dis* 2010, **2**(2):95-104.
35. Moldovan F, Pelletier JP, Jolicoeur FC, Cloutier JM, Martel-Pelletier J: **Diacerhein and rhein reduce the ICE-induced IL-1beta and IL-18 activation in human osteoarthritic cartilage.** *Osteoarthritis Cartilage* 2000, **8**(3):186-196.
36. Moore AR, Greenslade KJ, Alam CA, Willoughby DA: **Effects of diacerhein on granuloma induced cartilage breakdown in the mouse.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 1998, **6**(1):19-23.
37. Boittin M, Redini F, Loyau G, Pujol JP: **[Effect of diacerhein (ART 50) on the matrix synthesis and collagenase secretion by cultured joint chondrocytes in rabbits].** *Revue du rhumatisme* 1993, **60**(6 Pt 2):68S-76S.
38. Pelletier JP, Mineau F, Fernandes JC, Duval N, Martel-Pelletier J: **Diacerhein and rhein reduce the interleukin 1beta stimulated inducible nitric oxide synthesis level and activity while stimulating cyclooxygenase-2 synthesis in human osteoarthritic chondrocytes.** *J Rheumatol* 1998, **25**(12):2417-2424.
39. Dougados M, Nguyen M, Berdah L, Mazieres B, Vignon E, Lequesne M, Group EIS: **Evaluation of the structure-modifying effects of diacerein in hip osteoarthritis: ECHODIAH, a three-year, placebo-controlled trial. Evaluation of the Chondromodulating Effect of Diacerein in OA of the Hip.** *Arthritis and rheumatism* 2001, **44**(11):2539-2547.
40. Cohen SB, Proudman S, Kivitz AJ, Burch FX, Donohue JP, Burstein D, Sun YN, Banfield C, Vincent MS, Ni L *et al*: **A randomized, double-blind study of AMG 108 (a fully human monoclonal antibody to IL-1R1) in patients with osteoarthritis of the knee.** *Arthritis research & therapy* 2011, **13**(4):R125.
41. Chevalier X, Giraudeau B, Conrozier T, Marliere J, Kiefer P, Goupille P: **Safety study of intraarticular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: a multicenter study.** *The Journal of rheumatology* 2005, **32**(7):1317-1323.
42. Chevalier X, Goupille P, Beaulieu AD, Burch FX, Bensen WG, Conrozier T, Loeuille D, Kivitz AJ, Silver D, Appleton BE: **Intraarticular injection of anakinra in osteoarthritis of the knee: a multicenter, randomized, double-blind, placebo-controlled study.** *Arthritis and rheumatism* 2009, **61**(3):344-352.
43. Laine L: **Approaches to nonsteroidal anti-inflammatory drug use in the high-risk patient.** *Gastroenterology* 2001, **120**(3):594-606.
44. Gibofsky A, Hochberg MC, Jaros MJ, Young CL: **Efficacy and safety of low-dose submicron diclofenac for the treatment of osteoarthritis pain: a 12 week, phase 3 study.** *Current medical research and opinion* 2014, **30**(9):1883-1893.
45. Gallelli L, Galasso O, Falcone D, Southworth S, Greco M, Ventura V, Romualdi P, Corigliano A, Terracciano R, Savino R *et al*: **The effects of nonsteroidal anti-inflammatory drugs on clinical outcomes, synovial fluid cytokine concentration and signal transduction pathways in knee osteoarthritis. A randomized open label trial.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2013, **21**(9):1400-1408.
46. Bradley JD, Brandt KD, Katz BP, Kalasinski LA, Ryan SI: **Comparison of an antiinflammatory dose of ibuprofen, an analgesic dose of ibuprofen, and acetaminophen in the treatment of patients with osteoarthritis of the knee.** *The New England journal of medicine* 1991, **325**(2):87-91.
47. Wolfe MM, Lichtenstein DR, Singh G: **Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs.** *The New England journal of medicine* 1999, **340**(24):1888-1899.

48. Scheiman JM: **NSAIDs, gastrointestinal injury, and cytoprotection.** *Gastroenterology clinics of North America* 1996, **25**(2):279-298.
49. Kivitz A, Eisen G, Zhao WW, Bevirt T, Recker DP: **Randomized placebo-controlled trial comparing efficacy and safety of valdecoxib with naproxen in patients with osteoarthritis.** *The Journal of family practice* 2002, **51**(6):530-537.
50. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC *et al*: **Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group.** *The New England journal of medicine* 2000, **343**(21):1520-1528, 1522 p following 1528.
51. Singh G, Fort JG, Goldstein JL, Levy RA, Hanrahan PS, Bello AE, Andrade-Ortega L, Wallemark C, Agrawal NM, Eisen GM *et al*: **Celecoxib versus naproxen and diclofenac in osteoarthritis patients: SUCCESS-I Study.** *The American journal of medicine* 2006, **119**(3):255-266.
52. Mamdani M, Rochon P, Juurlink DN, Anderson GM, Kopp A, Naglie G, Austin PC, Laupacis A: **Effect of selective cyclooxygenase 2 inhibitors and naproxen on short-term risk of acute myocardial infarction in the elderly.** *Archives of internal medicine* 2003, **163**(4):481-486.
53. Jovanovic DV, Fernandes JC, Martel-Pelletier J, Jolicoeur FC, Reboul P, Laufer S, Tries S, Pelletier JP: **In vivo dual inhibition of cyclooxygenase and lipoxygenase by ML-3000 reduces the progression of experimental osteoarthritis: suppression of collagenase 1 and interleukin-1beta synthesis.** *Arthritis Rheum* 2001, **44**(10):2320-2330.
54. Pelletier JP, Boileau C, Brunet J, Boily M, Lajeunesse D, Reboul P, Laufer S, Martel-Pelletier J: **The inhibition of subchondral bone resorption in the early phase of experimental dog osteoarthritis by licofelone is associated with a reduction in the synthesis of MMP-13 and cathepsin K.** *Bone* 2004, **34**(3):527-538.
55. Raynauld JP, Martel-Pelletier J, Bias P, Laufer S, Haraoui B, Choquette D, Beaulieu AD, Abram F, Dorais M, Vignon E *et al*: **Protective effects of licofelone, a 5-lipoxygenase and cyclooxygenase inhibitor, versus naproxen on cartilage loss in knee osteoarthritis: a first multicentre clinical trial using quantitative MRI.** *Ann Rheum Dis* 2009, **68**(6):938-947.
56. Kulkarni SK, Singh VP: **Licofelone--a novel analgesic and anti-inflammatory agent.** *Current topics in medicinal chemistry* 2007, **7**(3):251-263.
57. Reijman M, Bierma-Zeinstra SM, Pols HA, Koes BW, Stricker BH, Hazes JM: **Is there an association between the use of different types of nonsteroidal antiinflammatory drugs and radiologic progression of osteoarthritis? The Rotterdam Study.** *Arthritis and rheumatism* 2005, **52**(10):3137-3142.
58. Chia WT, Pan RY, Tseng FJ, Chen YW, Feng CK, Lee HS, Chang DM, Sytwu HK: **Experimental osteoarthritis induced by surgical realignment of the patella in BALB/c mice.** *JBone Joint SurgBr* 2010, **92**(12):1710-1716.
59. Bentz M, Zaouter C, Shi Q, Fahmi H, Moldovan F, Fernandes JC, Benderdour M: **Inhibition of inducible nitric oxide synthase prevents lipid peroxidation in osteoarthritic chondrocytes.** *Journal of cellular biochemistry* 2012, **113**(7):2256-2267.
60. Hashimoto S, Takahashi K, Amiel D, Coutts RD, Lotz M: **Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis.** *Arthritis and rheumatism* 1998, **41**(7):1266-1274.
61. Scher J, Pillinger M, Abramson S: **Nitric oxide synthases and osteoarthritis.** *Current rheumatology reports* 2007, **9**(1):9-15.
62. Jarvinen K, Vuolteenaho K, Nieminen R, Moilanen T, Knowles RG, Moilanen E: **Selective iNOS inhibitor 1400W enhances anti-catabolic IL-10 and reduces destructive MMP-10 in OA cartilage. Survey of the effects of 1400W on inflammatory mediators produced by OA cartilage as detected by protein antibody array.** *Clinical and experimental rheumatology* 2008, **26**(2):275-282.

63. Salerno L, Sorrenti V, Di Giacomo C, Romeo G, Siracusa MA: **Progress in the development of selective nitric oxide synthase (NOS) inhibitors.** *Current pharmaceutical design* 2002, **8**(3):177-200.
64. More AS, Kumari RR, Gupta G, Lingaraju MC, Balaganur V, Pathak NN, Kumar D, Sharma AK, Tandan SK: **Effect of iNOS inhibitor S-methylisothiourea in monosodium iodoacetate-induced osteoarthritic pain: implication for osteoarthritis therapy.** *Pharmacology, biochemistry, and behavior* 2013, **103**(4):764-772.
65. Pelletier JP, Jovanovic D, Fernandes JC, Manning P, Connor JR, Currie MG, Martel-Pelletier J: **Reduction in the structural changes of experimental osteoarthritis by a nitric oxide inhibitor.** *Osteoarthritis and Cartilage* 1999, **7**(4):416-418.
66. Hellio le Graverand MP, Clemmer RS, Redifer P, Brunell RM, Hayes CW, Brandt KD, Abramson SB, Manning PT, Miller CG, Vignon E: **A 2-year randomised, double-blind, placebo-controlled, multicentre study of oral selective iNOS inhibitor, cindunistat (SD-6010), in patients with symptomatic osteoarthritis of the knee.** *Annals of the rheumatic diseases* 2013, **72**(2):187-195.
67. More AS, Kumari RR, Gupta G, Lingaraju MC, Balaganur V, Pathak NN, Kumar D, Kumar D, Sharma AK, Tandan SK: **Effect of iNOS inhibitor S-methylisothiourea in monosodium iodoacetate-induced osteoarthritic pain: Implication for osteoarthritis therapy.** *Pharmacology Biochemistry and Behavior* 2013, **103**(4):764-772.
68. Mobasheri A: **The future of osteoarthritis therapeutics: targeted pharmacological therapy.** *Curr Rheumatol Rep* 2013, **15**(10):364.
69. Sohn DH, Sokolove J, Sharpe O, Erhart JC, Chandra PE, Lahey LJ, Lindstrom TM, Hwang I, Boyer KA, Andriacchi TP *et al*: **Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4.** *Arthritis Res Ther* 2012, **14**(1):R7.
70. Scanzello CR, Umoh E, Pessler F, Diaz-Torne C, Miles T, Dicarlo E, Potter HG, Mandl L, Marx R, Rodeo S *et al*: **Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stage disease.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2009, **17**(8):1040-1048.
71. Ling SM, Patel DD, Garnerio P, Zhan M, Vaduganathan M, Muller D, Taub D, Bathon JM, Hochberg M, Abernethy DR *et al*: **Serum protein signatures detect early radiographic osteoarthritis.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2009, **17**(1):43-48.
72. Sun JM, Sun LZ, Liu J, Su BH, Shi L: **Serum interleukin-15 levels are associated with severity of pain in patients with knee osteoarthritis.** *Disease markers* 2013, **35**(3):203-206.
73. Hartgring SA, van Roon JA, Wenting-van Wijk M, Jacobs KM, Jahangier ZN, Willis CR, Bijlsma JW, Lafeber FP: **Elevated expression of interleukin-7 receptor in inflamed joints mediates interleukin-7-induced immune activation in rheumatoid arthritis.** *Arthritis and rheumatism* 2009, **60**(9):2595-2605.
74. van Roon JA, Glaudemans KA, Bijlsma JW, Lafeber FP: **Interleukin 7 stimulates tumour necrosis factor alpha and Th1 cytokine production in joints of patients with rheumatoid arthritis.** *Annals of the rheumatic diseases* 2003, **62**(2):113-119.
75. Long D, Blake S, Song XY, Lark M, Loeser RF: **Human articular chondrocytes produce IL-7 and respond to IL-7 with increased production of matrix metalloproteinase-13.** *Arthritis research & therapy* 2008, **10**(1):R23.
76. Han L, Lee HS, Yoon JH, Choi WS, Park YG, Nam SW, Lee JY, Park WS: **Association of IL-17A and IL-17F single nucleotide polymorphisms with susceptibility to osteoarthritis in a Korean population.** *Gene* 2014, **533**(1):119-122.
77. Chen B, Deng Y, Tan Y, Qin J, Chen LB: **Association between severity of knee osteoarthritis and serum and synovial fluid interleukin 17 concentrations.** *The Journal of international medical research* 2014, **42**(1):138-144.

78. Martel-Pelletier J, Mineau F, Jovanovic D, Di Battista JA, Pelletier JP: **Mitogen-activated protein kinase and nuclear factor kappaB together regulate interleukin-17-induced nitric oxide production in human osteoarthritic chondrocytes: possible role of transactivating factor mitogen-activated protein kinase-activated protein kinase (MAPKAPK).** *Arthritis Rheum* 1999, **42**(11):2399-2409.
79. Tanigawa S, Aida Y, Kawato T, Honda K, Nakayama G, Motohashi M, Suzuki N, Ochiai K, Matsumura H, Maeno M: **Interleukin-17F affects cartilage matrix turnover by increasing the expression of collagenases and stromelysin-1 and by decreasing the expression of their inhibitors and extracellular matrix components in chondrocytes.** *Cytokine* 2011, **56**(2):376-386.
80. Zhou Y, Millward-Sadler SJ, Lin H, Robinson H, Goldring M, Salter DM, Nuki G: **Evidence for JNK-dependent up-regulation of proteoglycan synthesis and for activation of JNK1 following cyclical mechanical stimulation in a human chondrocyte culture model.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2007, **15**(8):884-893.
81. Yasuda T, Julovi SM, Hiramitsu T, Yoshida M, Nakamura T: **Requirement of mitogen-activated protein kinase for collagenase production by the fibronectin fragment in human articular chondrocytes in culture.** *Modern rheumatology / the Japan Rheumatism Association* 2004, **14**(1):54-60.
82. Brown KK, Heitmeyer SA, Hookfin EB, Hsieh L, Buchalova M, Taiwo YO, Janusz MJ: **P38 MAP kinase inhibitors as potential therapeutics for the treatment of joint degeneration and pain associated with osteoarthritis.** *J Inflamm (Lond)* 2008, **5**:22.
83. Pelletier JP, Fernandes JC, Brunet J, Moldovan F, Schrier D, Flory C, Martel-Pelletier J: **In vivo selective inhibition of mitogen-activated protein kinase kinase 1/2 in rabbit experimental osteoarthritis is associated with a reduction in the development of structural changes.** *Arthritis Rheum* 2003, **48**(6):1582-1593.
84. Boileau C, Martel-Pelletier J, Brunet J, Schrier D, Flory C, Boily M, Pelletier JP: **PD-0200347, an alpha2delta ligand of the voltage gated calcium channel, inhibits in vivo activation of the Erk1/2 pathway in osteoarthritic chondrocytes: a PKCalpha dependent effect.** *Ann Rheum Dis* 2006, **65**(5):573-580.
85. Badger AM, Griswold DE, Kapadia R, Blake S, Swift BA, Hoffman SJ, Stroup GB, Webb E, Rieman DJ, Gowen M *et al*: **Disease-modifying activity of SB 242235, a selective inhibitor of p38 mitogen-activated protein kinase, in rat adjuvant-induced arthritis.** *Arthritis and rheumatism* 2000, **43**(1):175-183.
86. van den Blink B, Juffermans NP, ten Hove T, Schultz MJ, van Deventer SJ, van der Poll T, Peppelenbosch MP: **p38 mitogen-activated protein kinase inhibition increases cytokine release by macrophages in vitro and during infection in vivo.** *J Immunol* 2001, **166**(1):582-587.
87. Gaestel M: **MAPKAP kinases - MKs - two's company, three's a crowd.** *Nature reviews Molecular cell biology* 2006, **7**(2):120-130.
88. Kotlyarov A, Neininger A, Schubert C, Eckert R, Birchmeier C, Volk HD, Gaestel M: **MAPKAP kinase 2 is essential for LPS-induced TNF-alpha biosynthesis.** *Nature cell biology* 1999, **1**(2):94-97.
89. Jones SW, Brockbank SM, Clements KM, Le Good N, Campbell D, Read SJ, Needham MR, Newham P: **Mitogen-activated protein kinase-activated protein kinase 2 (MK2) modulates key biological pathways associated with OA disease pathology.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2009, **17**(1):124-131.
90. Liu X, Wu T, Chi P: **Inhibition of MK2 shows promise for preventing postoperative ileus in mice.** *The Journal of surgical research* 2013, **185**(1):102-112.
91. Bobacz K, Sunk IG, Hofstaetter JG, Amoyo L, Toma CD, Akira S, Weichhart T, Saemann M, Smolen JS: **Toll-like receptors and chondrocytes: the lipopolysaccharide-induced decrease in cartilage matrix synthesis is dependent on the presence of toll-like receptor 4 and**

- antagonized by bone morphogenetic protein 7. *Arthritis and rheumatism* 2007, **56**(6):1880-1893.
92. Kim HA, Cho ML, Choi HY, Yoon CS, Jhun JY, Oh HJ, Kim HY: **The catabolic pathway mediated by Toll-like receptors in human osteoarthritic chondrocytes.** *Arthritis and rheumatism* 2006, **54**(7):2152-2163.
 93. Sillat T, Barreto G, Clarijs P, Soininen A, Ainola M, Pajarinen J, Korhonen M, Konttinen YT, Sakalyte R, Hukkanen M *et al*: **Toll-like receptors in human chondrocytes and osteoarthritic cartilage.** *Acta orthopaedica* 2013, **84**(6):585-592.
 94. Gomez R, Villalvilla A, Largo R, Gualillo O, Herrero-Beaumont G: **TLR4 signalling in osteoarthritis--finding targets for candidate DMOADs.** *Nature reviews Rheumatology* 2015, **11**(3):159-170.
 95. Campo GM, Avenoso A, D'Ascola A, Scuruchi M, Prestipino V, Nastasi G, Calatroni A, Campo S: **Adenosine A2A receptor activation and hyaluronan fragment inhibition reduce inflammation in mouse articular chondrocytes stimulated with interleukin-1beta.** *The FEBS journal* 2012, **279**(12):2120-2133.
 96. Wang Q, Pan X, Wong HH, Wagner CA, Lahey LJ, Robinson WH, Sokolove J: **Oral and topical boswellic acid attenuates mouse osteoarthritis.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2014, **22**(1):128-132.
 97. Sacre SM, Andreakos E, Kiriakidis S, Amjadi P, Lundberg A, Giddins G, Feldmann M, Brennan F, Foxwell BM: **The Toll-like receptor adaptor proteins MyD88 and Mal/TIRAP contribute to the inflammatory and destructive processes in a human model of rheumatoid arthritis.** *The American journal of pathology* 2007, **170**(2):518-525.
 98. Ehrentraut H, Meyer R, Schwederski M, Ehrentraut S, Velten M, Grohe C, Knuefermann P, Baumgarten G, Boehm O: **Systemically administered ligands of Toll-like receptor 2, -4, and -9 induce distinct inflammatory responses in the murine lung.** *Mediators of inflammation* 2011, **2011**:746532.
 99. Komai-Koma M, Li D, Wang E, Vaughan D, Xu D: **Anti-Toll-like receptor 2 and 4 antibodies suppress inflammatory response in mice.** *Immunology* 2014, **143**(3):354-362.
 100. Blom AB, van Lent, P.L., Abdollahi-Roodsaz, S., van de Kraan, P., van den Berg, W.: **Elusive Role for Toll Like receptor 2 in Joint Pathology in Experimental Osteoarthritis.** *Osteoarthritis and Cartilage* 2011, **19**(Supplement 1):S18.
 101. Kershaw EE, Flier JS: **Adipose tissue as an endocrine organ.** *The Journal of clinical endocrinology and metabolism* 2004, **89**(6):2548-2556.
 102. Deng Y, Scherer PE: **Adipokines as novel biomarkers and regulators of the metabolic syndrome.** *Annals of the New York Academy of Sciences* 2010, **1212**:E1-E19.
 103. Richette P, Poitou C, Garnero P, Vicaut E, Bouillot JL, Lacorte JM, Basdevant A, Clement K, Bardin T, Chevalier X: **Benefits of massive weight loss on symptoms, systemic inflammation and cartilage turnover in obese patients with knee osteoarthritis.** *Ann Rheum Dis* 2011, **70**(1):139-144.
 104. Gomez R, Lago F, Gomez-Reino J, Dieguez C, Gualillo O: **Adipokines in the skeleton: influence on cartilage function and joint degenerative diseases.** *Journal of molecular endocrinology* 2009, **43**(1):11-18.
 105. Thijssen E, van Caam A, van der Kraan PM: **Obesity and osteoarthritis, more than just wear and tear: pivotal roles for inflamed adipose tissue and dyslipidaemia in obesity-induced osteoarthritis.** *Rheumatology (Oxford)* 2015, **54**(4):588-600.
 106. Cicuttini FM, Baker JR, Spector TD: **The association of obesity with osteoarthritis of the hand and knee in women: a twin study.** *J Rheumatol* 1996, **23**(7):1221-1226.
 107. Friedman JM, Halaas JL: **Leptin and the regulation of body weight in mammals.** *Nature* 1998, **395**(6704):763-770.

108. Ku JH, Lee CK, Joo BS, An BM, Choi SH, Wang TH, Cho HL: **Correlation of synovial fluid leptin concentrations with the severity of osteoarthritis.** *Clinical rheumatology* 2009, **28**(12):1431-1435.
109. Hui W, Litherland GJ, Elias MS, Kitson GI, Cawston TE, Rowan AD, Young DA: **Leptin produced by joint white adipose tissue induces cartilage degradation via upregulation and activation of matrix metalloproteinases.** *Annals of the rheumatic diseases* 2012, **71**(3):455-462.
110. Vuolteenaho K, Koskinen A, Kukkonen M, Nieminen R, Paivarinta U, Moilanen T, Moilanen E: **Leptin enhances synthesis of proinflammatory mediators in human osteoarthritic cartilage--mediator role of NO in leptin-induced PGE2, IL-6, and IL-8 production.** *Mediators of inflammation* 2009, **2009**:345838.
111. Gertler A: **Development of leptin antagonists and their potential use in experimental biology and medicine.** *Trends in endocrinology and metabolism: TEM* 2006, **17**(9):372-378.
112. Babaei A, Zarkesh-Esfahani SH, Bahrami E, Ross RJ: **Restricted leptin antagonism as a therapeutic approach to treatment of autoimmune diseases.** *Hormones (Athens)* 2011, **10**(1):16-26.
113. Scherer LJ, Rossi JJ: **Approaches for the sequence-specific knockdown of mRNA.** *Nature biotechnology* 2003, **21**(12):1457-1465.
114. Jones SW, Souza PM, Lindsay MA: **siRNA for gene silencing: a route to drug target discovery.** *Current opinion in pharmacology* 2004, **4**(5):522-527.
115. Turner JJ, Jones SW, Moschos SA, Lindsay MA, Gait MJ: **MALDI-TOF mass spectral analysis of siRNA degradation in serum confirms an RNase A-like activity.** *Molecular bioSystems* 2007, **3**(1):43-50.
116. Moschos SA, Jones SW, Perry MM, Williams AE, Erjefalt JS, Turner JJ, Barnes PJ, Sproat BS, Gait MJ, Lindsay MA: **Lung delivery studies using siRNA conjugated to TAT(48-60) and penetratin reveal peptide induced reduction in gene expression and induction of innate immunity.** *Bioconjugate chemistry* 2007, **18**(5):1450-1459.
117. Thakur A, Fitzpatrick S, Zaman A, Kugathasan K, Muirhead B, Hortelano G, Sheardown H: **Strategies for ocular siRNA delivery: Potential and limitations of non-viral nanocarriers.** *Journal of biological engineering* 2012, **6**(1):7.
118. Komano Y, Yagi N, Onoue I, Kaneko K, Miyasaka N, Nanki T: **Arthritic joint-targeting small interfering RNA-encapsulated liposome: implication for treatment strategy for rheumatoid arthritis.** *J Pharmacol Exp Ther* 2012, **340**(1):109-113.
119. Courties G, Baron M, Presumey J, Escriou V, van Lent P, Scherman D, Cantagrel A, van den Berg WB, Jorgensen C, Apparailly F et al: **Cytosolic phospholipase A2alpha gene silencing in the myeloid lineage alters development of Th1 responses and reduces disease severity in collagen-induced arthritis.** *Arthritis and rheumatism* 2011, **63**(3):681-690.
120. Chen LX, Lin L, Wang HJ, Wei XL, Fu X, Zhang JY, Yu CL: **Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF-kappaBp65-specific siRNA.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2008, **16**(2):174-184.
121. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP et al: **Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals.** *Nature* 2009, **458**(7235):223-227.
122. Brown TD, Johnston RC, Saltzman CL, Marsh JL, Buckwalter JA: **Posttraumatic osteoarthritis: a first estimate of incidence, prevalence, and burden of disease.** *Journal of orthopaedic trauma* 2006, **20**(10):739-744.
123. Soren A, Klein W, Huth F: **The synovial changes in post-traumatic synovitis and osteoarthritis.** *Rheumatology and rehabilitation* 1978, **17**(1):38-45.

124. Cameron ML, Fu FH, Paessler HH, Schneider M, Evans CH: **Synovial fluid cytokine concentrations as possible prognostic indicators in the ACL-deficient knee.** *Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA* 1994, **2**(1):38-44.
125. Irie K, Uchiyama E, Iwaso H: **Intraarticular inflammatory cytokines in acute anterior cruciate ligament injured knee.** *The Knee* 2003, **10**(1):93-96.
126. Presle N, Pottie P, Dumond H, Guillaume C, Lapique F, Pallu S, Mainard D, Netter P, Terlain B: **Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production.** *Osteoarthritis Cartilage* 2006, **14**(7):690-695.
127. Holliday KL, McWilliams DF, Maciewicz RA, Muir KR, Zhang W, Doherty M: **Lifetime body mass index, other anthropometric measures of obesity and risk of knee or hip osteoarthritis in the GOAL case-control study.** *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2011, **19**(1):37-43.
128. Maillfert JF, Gueguen A, Monreal M, Nguyen M, Berdah L, Lequesne M, Mazieres B, Vignon E, Dougados M: **Sex differences in hip osteoarthritis: results of a longitudinal study in 508 patients.** *Annals of the rheumatic diseases* 2003, **62**(10):931-934.
129. Srikanth VK, Fryer JL, Zhai G, Winzenberg TM, Hosmer D, Jones G: **A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis.** *Osteoarthritis Cartilage* 2005, **13**(9):769-781.
130. Nadkar MY, Samant RS, Vaidya SS, Borges NE: **Relationship between osteoarthritis of knee and menopause.** *J Assoc Physicians India* 1999, **47**(12):1161-1163.
131. Cirillo DJ, Wallace RB, Wu L, Yood RA: **Effect of hormone therapy on risk of hip and knee joint replacement in the Women's Health Initiative.** *Arthritis Rheum* 2006, **54**(10):3194-3204.
132. Spector TD, Nandra D, Hart DJ, Doyle DV: **Is hormone replacement therapy protective for hand and knee osteoarthritis in women?: The Chingford Study.** *Ann Rheum Dis* 1997, **56**(7):432-434.
133. Bergink AP, van Meurs JB, Loughlin J, Arp PP, Fang Y, Hofman A, van Leeuwen JP, van Duijn CM, Uitterlinden AG, Pols HA: **Estrogen receptor alpha gene haplotype is associated with radiographic osteoarthritis of the knee in elderly men and women.** *Arthritis Rheum* 2003, **48**(7):1913-1922.
134. Maneix L, Beauchef G, Servent A, Wegrowski Y, Maquart FX, Boujrad N, Flouriot G, Pujol JP, Boumediene K, Galera P *et al*: **17Beta-oestradiol up-regulates the expression of a functional UDP-glucose dehydrogenase in articular chondrocytes: comparison with effects of cytokines and growth factors.** *Rheumatology (Oxford)* 2008, **47**(3):281-288.
135. Morisset S, Patry C, Lora M, de Brum-Fernandes AJ: **Regulation of cyclooxygenase-2 expression in bovine chondrocytes in culture by interleukin 1alpha, tumor necrosis factor-alpha, glucocorticoids, and 17beta-estradiol.** *J Rheumatol* 1998, **25**(6):1146-1153.
136. Oestergaard S, Sondergaard BC, Hoegh-Andersen P, Henriksen K, Qvist P, Christiansen C, Tanko LB, Karsdal MA: **Effects of ovariectomy and estrogen therapy on type II collagen degradation and structural integrity of articular cartilage in rats: implications of the time of initiation.** *Arthritis Rheum* 2006, **54**(8):2441-2451.
137. Claassen H, Schunke M, Kurz B: **Estradiol protects cultured articular chondrocytes from oxygen-radical-induced damage.** *Cell Tissue Res* 2005, **319**(3):439-445.
138. Cutolo M: **Selective estrogen receptor agonism lacks clinical benefit in rheumatoid arthritis: comment on the article by van Vollenhoven et al.** *Arthritis Rheum* 2010, **62**(12):3832-3833.
139. Armour AA, Watkins CL: **The challenge of targeting EGFR: experience with gefitinib in nonsmall cell lung cancer.** *European respiratory review : an official journal of the European Respiratory Society* 2010, **19**(117):186-196.

140. Rushton MD, Young DA, Loughlin J, Reynard LN: **Differential DNA methylation and expression of inflammatory and zinc transporter genes defines subgroups of osteoarthritic hip patients.** *Annals of the rheumatic diseases* 2015.
141. Shin K, Kim JW, Moon KW, Yang JA, Lee EY, Song YW, Lee EB: **The efficacy of diacerein in hand osteoarthritis: a double-blind, randomized, placebo-controlled study.** *Clinical therapeutics* 2013, **35**(4):431-439.
142. Schnitzer TJ, Dattani ID, Serio B, Schneider H, Moore A, Tseng L, Sallstig P, Rebuli R, Maxwell T: **A 13-week, multicenter, randomized, double-blind study of lumiracoxib in hip osteoarthritis.** *Clinical rheumatology* 2011, **30**(11):1433-1446.
143. Lago R, Gomez R, Otero M, Lago F, Gallego R, Dieguez C, Gomez-Reino JJ, Gualillo O: **A new player in cartilage homeostasis: adiponectin induces nitric oxide synthase type II and pro-inflammatory cytokines in chondrocytes.** *Osteoarthritis and Cartilage* 2008, **16**(9):1101-1109.
144. Abdollahi-Roodsaz S, Joosten LA, Koenders MI, van den Brand BT, van de Loo FA, van den Berg WB: **Local interleukin-1-driven joint pathology is dependent on toll-like receptor 4 activation.** *The American journal of pathology* 2009, **175**(5):2004-2013.
145. Berry PA JS, Cicuttini FM, Wluka AE, Maciewicz RA.: **Temporal relationship between serum adipokines, biomarkers of bone and cartilage turnover, and cartilage volume loss in a population with clinical knee osteoarthritis.** *Arthritis and rheumatism* 2011, **63**(3):700-707.
146. Busso N, So A, Chobaz-Peclat V, Morard C, Martinez-Soria E, Talabot-Ayer D, Gabay C: **Leptin signaling deficiency impairs humoral and cellular immune responses and attenuates experimental arthritis.** *J Immunol* 2002, **168**(2):875-882.

932 **Table 1: Completed RCTs of anti-inflammatory therapeutics in OA**

Target Class	Mode-of-action	Drug	Clinical Trial Data			
			Phase	Patient Cohort	Clinical End Point	Results
TNFα inhibition	TNF α mAb	Adalimumab	PhII (NCT00296894) [19]	Hand OA (60 patients)	Incidence of new erosive interphalangeal joint	n/s vs placebo
			PhI/II (NCT00686439) [20]	Knee OA (17 patients)	Pain (WOMAC).	Reduction in joint pain
			PhII (NCT00597623) [21]	Refractory hand OA (99 patients)	Pain.	n/s vs placebo
		DLX105	PhI/IIa (NCT00819572)	Painful knee OA (27 patients)	Pain (VAS)	Completed 2010, no results published.
IL1β inhibition	IL1 β small molecule pathway inhibitor	Diacerein	PhII (NCT00451360) [39]	Hip OA (507 patients)	Pain, function and JSN.	Significant reduction in JSN vs placebo.
			PhIV (NCT00685542) [141]	Hand OA (86 patients)	Pain (AUSCAN)	n/s vs placebo
	IL-1R1 mAb	AMG108	PhII (NCT00110942) [40]	Knee OA (160 patients)	Pain. No disease modification end-point.	n/s vs placebo
	IL1 β recombinant protein inhibitor (i.a)	Anakinra	PhII (NCT00110916) [42]	Painful knee OA (170 patients)	Pain, stiffness and physical function (WOMAC). Cartilage turnover.	n/s vs placebo
Arachidonic acid pathway inhibitors	COX-2 inhibitor	Valdecoxib	PhII (NCT00650624) [49]	Symptomatic OA of the knee (1019 patients)	Pain (WOMAC)	Significant reduction in pain
		Lumiracoxib	PhIII (NCT00154219) [142]	Primary Hip OA (1262 patients)	Pain (VAS)	Significant reduction in VAS score and improvement in function
	5-lipoxygenase and cyclo-oxygenase inhibitor	Licofelone	Unknown [55]	Knee OA (355 patients)	Cartilage volume and JSN by assessed by quantitative MRI	Significant reduced cartilage volume loss. JSN remained unchanged
NO inhibitors	iNOS inhibitor	SD-6010	PhII/III (NCT00565812) [66]	Overweight patients with knee OA (1457 patients)	JSN	n/s vs placebo

933 Abbreviations: Ph, Phase; n/s, non-significant; WOMAC, Western Ontario and
934 McMaster Universities Arthritis Index; OA, osteoarthritis; JSN, joint space narrowing;
935 VAS, Visual Analogue Scale; NO, Nitric Oxide; IL1 β , Interleukin-1 Beta; TNF α ,
936 Tumour Necrosis Factor alpha; COX-2, cyclooxygenase-2; AUSCAN,
937 Australian/Canadian Hand Osteoarthritis Index.

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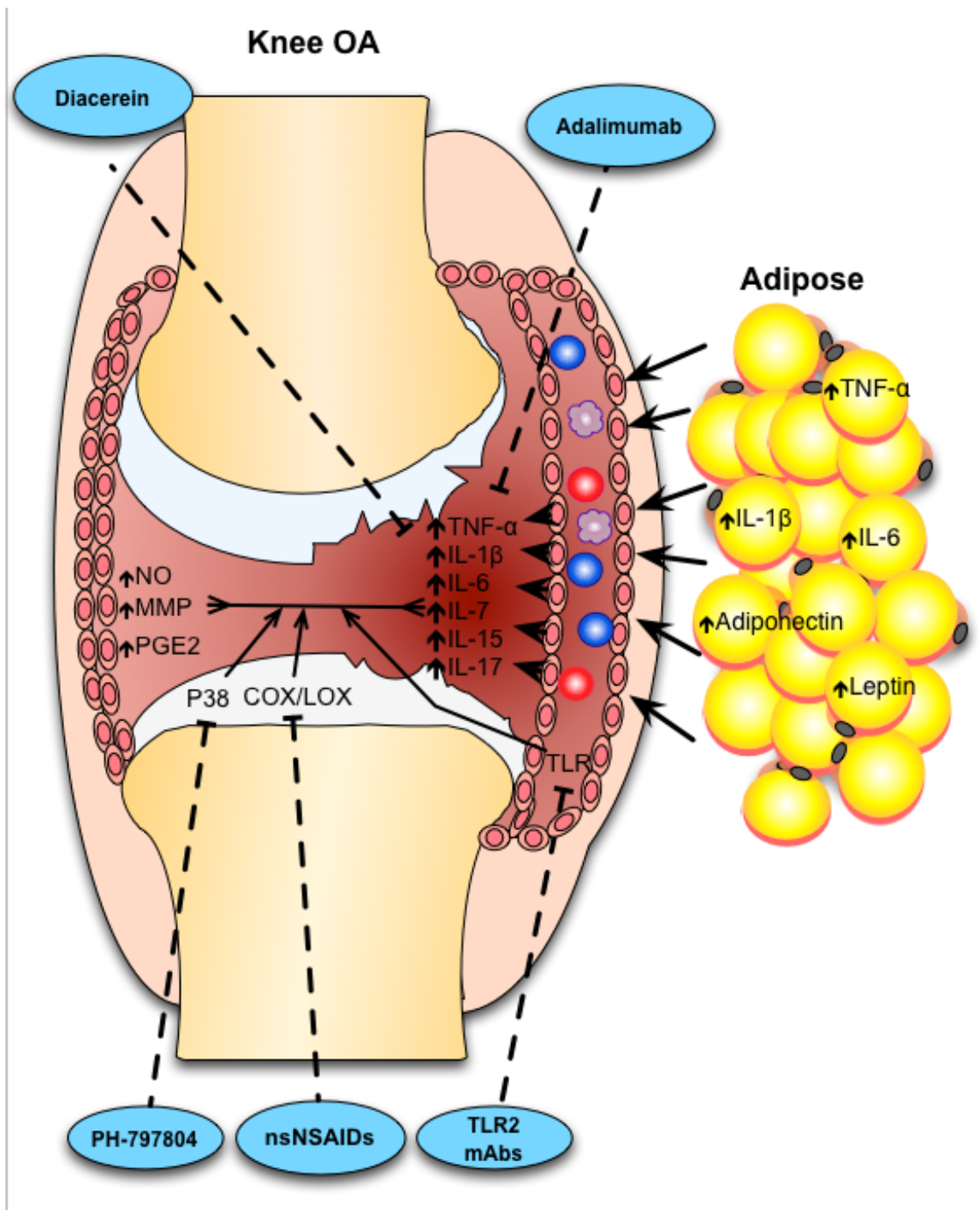
Table 2. Promising emerging targets for development of a DMOAD

Target Class	Target	Disease Linkage	Preclinical Validation	
			<i>In vitro</i>	<i>In vivo</i>
Cytokines and chemokines	IL7	Expressed by articular chondrocytes [75]	Induces MMP13 and proteoglycan loss in cartilage [75]	
	IL-15	Elevated in synovial fluid of patients with early OA [143]. Serum levels associated with knee OA progression [71] and pain [72].		
	IL-17	Polymorphisms correlate to OA susceptibility [76]. Elevated in both serum and synovial fluid in knee OA, and correlation to disease severity [77].	Stimulates production of NO and MMPs in human chondrocytes [78], and reduced TIMP expression [79].	
p38 pathway inhibitors	p38 MAPK	P38 activated by OA relevant pro-inflammatory cytokines	p38 inhibition blocks cytokine mediated production of PGE2, MMP3 and MMP13 in human chondrocytes [89]	p38 inhibition reduced in cartilage loss in score in MIA OA model [82-84]
	MAPKAP K2 (MK2)	Active phospho MK2 in areas of OA cartilage damage.	MK2 inhibition blocks cytokine mediated production of PGE2, MMP3 and MMP13 in human chondrocytes [89]	
Toll-like receptors	TLR2	Activated by DAMPS in OA synovial fluid. Expression high in areas of cartilage damage [92].	TLR2 induces cytokine production [97-99] and cartilage damage [92].	TLR2 mAbs reduce disease score in CIA model [99]
	TLR4	Activated by DAMPS in OA synovial fluid. Expression high in areas of cartilage damage [92], and increased with JSN progression [93].	Activation promotes cytokine production in human chondrocytes [144]. Non-specific TLR4 inhibitors reduce pro-catabolic effects in chondrocytes [91, 95]	TLR4 KO mice protected against IL-1 β mediated OA [144]. Non-specific TLR4 inhibitors protective for cartilage loss in murine OA model [96]
Adipokines	Leptin	Elevated in OA serum or synovial fluid [108]. Correlation between serum levels and cartilage loss at 2 years [145]	Induces production of MMPs [109] and pro-inflammatory cytokines [110] in chondrocytes.	Leptin deficient mice (ob/ob) are protected from experimentally induced arthritis [146]
RNAi therapeutics	TNF α (siRNA)	Elevated levels of TNF α in OA serum and synovial fluid [9-11]	Inhibition of TNF- α production	siRNA reduced disease score in CIA murine model [118]
	Pan-cytokine (siRNA)	Elevated levels of pro-inflammatory cytokines in OA synovial fluid [4, 69]	Inhibition of TNF- α , IL-1 β , IL-6, IL-18 and A2 α production [118]	siRNA reduced disease score in CIA murine model [118]

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950 Abbreviations: MMP, matrix metalloproteinase; OA, osteoarthritis; MAPK, mitogen
951 activated protein kinase; DAMPS, damage associated molecular pattern molecules;
952 siRNA, small interfering RNA; TLR, toll like receptor; KO, knockout; NO, nitric oxide;
953 JSN, joint space narrowing.


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956 **Figure 1: The relationship between joint inflammation and OA pathology.**

957 Cartilage degradation and fragmentation causes synovial inflammation, and
 958 increases production of pro-degradative (MMPs) and pro-inflammatory (IL-6, IL7,
 959 IL1β, IL-15, IL-17, TNFα) proteins. Synovium immune cell infiltration and obese
 960 adipose tissue contribute towards increased production of pro-inflammatory
 961 cytokines and thus increase the production of proteolytic enzymes which drive
 962 further cartilage breakdown. Blue ovals represent current and potential drugs and

963 their associated targets.  = Immune cells including B cells, T cells, and
 964 macrophages. Abbreviations; IL, interleukin; MMP, matrix metalloproteinase; NO,

965 nitric oxide; OA, osteoarthritis; PGE2, prostaglandin E2; TNF, tumour necrosis factor;
966 TLR: Toll-like receptor.
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